



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

GAU 1643  
PHS  
**RECEIVED**

FEB 22 2001

TECH CENTER 1600/2900

**Applicant:** David A. Tirrell et al.  
**Serial No.:** 09/620,691  
**Filed:** July 20, 2000  
**Docket:** 30431.5US01  
**Title:** METHODS FOR STABILIZATION OF PROTEINS USING NON-NATURAL AMINO ACIDS

CERTIFICATE UNDER 37 CFR 1.8

I hereby certify that this paper or fee is being deposited with the United States Postal as first class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 on February 6, 2001.

By: [Signature]  
Name: Richelle Ann Domingo

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

We are transmitting herewith the attached:

- ☒ Transmittal sheet, in duplicate, containing Certificate under 37 CFR 1.8.
- ☒ Information Disclosure Statement (37 C.F.R. §1.97 (d))
- ☒ Form 1449 (Information Disclosure Statement)
- ☒ Exhibits 1-58
- ☒ Return postcard

Please charge any additional fees or credit overpayment to Deposit Account No. 50-0306. A duplicate of this sheet is enclosed.

**MANDEL & ADRIANO**

35 No. Arroyo Parkway, Suite 60  
Pasadena, California 91103  
(626)395-7801

By: Sarah B. Adriano

Name: Sarah B. Adriano  
Reg. No.: 34,470  
Initials: SBA  
Customer No.: 26,941

**RECEIVED**

FEB 22 2001

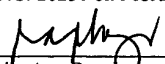
TECH CENTER 1600/2900

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**Applicant:** David A. Tirrell, et al. **Examiner:** Not Yet Known  
**Serial No.:** 09/620,691 **Group Art Unit:** 1643  
**Filed:** July 20, 2000 **Docket No.:** 30431.5US01  
**Title:** METHODS FOR STABILIZATION OF PROTEINS USING NON-NATURAL AMINO ACIDS

**CERTIFICATE UNDER 37 CFR 1.8:**

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on February 6, 2001.

  
By: Richelle Ann Domingo

**INFORMATION DISCLOSURE STATEMENT (37 C.F.R. §1.97(d))**

Assistant Commissioner for Patents  
Washington, D.C. 20231  
February 6, 2001

Dear Sir:

With regard to the above-identified application, the items of information listed on the enclosed Form 1449 are brought to the attention of the Examiner. They are as follows:

- Aizawa, Y. et al., "Stability of the Dimerization Domain Effects the Cooperative DNA Binding of Short Peptides", *Biochemistry* (1999), 38, 4008-4017. (Exhibit 1)
- Arndt, K. et al., "GCN4 protein, a positive transcription factor in yeast, binds general control promoters at all 5' TGACTC 3' sequences", *Proc. Natl. Acad. Sci. USA*, (1986), 83, 8516-8520. (Exhibit 2)
- Baldwin, E. et al., "Thermodynamic and Structural Compensation in "Size-switch" Core Repacking Variants of Bacteriophage T4 Lysozyme", *J. Mol. Biol.* (1996), 259: 542-559. (Exhibit 3)
- Chao, H. et al., "Kinetic Study on the Formation of a de Novo Designed Heterodimeric Coiled-Coil: Use of Surface Plasmon Resonance to Monitor the Association and Dissociation of Polypeptide Chains:", *Biochemistry* (1996), 35: 12175-12185. (Exhibit 4)

- Chenault, H. K. et al., "Kinetic Resolution of Unsaturated and Rarely Occuring Samino Acids: Enantioselective Hydrolysis of N-Acyl Amino Acids Catalyzed by Acylase I", *J. Am. Chem. Soc.* (1989), 111, 6354-6364. (Exhibit 5)
- Cornish, V. M. et al., "Probing Protein Structure and Function with an Expanded Genetic Code", *Angew. Chem. Int. Ed. Engl.* (1995), 34: 621-633. (Exhibit 6)
- d'Avignon, D. A. et al., "Thermodynamic and kinetics of a folded-folded transition at Valine-9 of a GCN4-like leucine zipper", *Biophys. J.* (1999), 76: 2752-2759. (Exhibit 7)
- Dahiyat, B.L. et al., "De Novo Protein Design: Fully Automated Sequence Selection" *Science* (1997), 278: 82-87. (Exhibit 8)
- DeGrado, W. F. et al., "De Novo Design and Structural Characterization of Proteins and Metalloproteins", *Annu. Rev. Biochem.* (1999), 68: 779-819. (Exhibit 9)
- Duewel, H. et al., "Incorporation of Trifluoromethionine into a Phage Lysozyme: Implication and a New Marker for Use in Protein 19F NMR", *Biochemistry* (1997), 36, 3404-3416. (Exhibit 10)
- Ellenberger, T. E. et al., "The GCN4 Basic Region Leucine Zipper Binds DNA as a Dimer of Uninterrupted  $\alpha$  Helices: Crystal Structure of the Protein-DNA Complex" *Cell* (1992), 71: 1223-1237. (Exhibit 11)
- Fersht, A. R. et al., "Principles of protein stability derived from protein engineering experiments", *Curr. Opin. Struct. Biol.* (1993), 3: 75-83. (Exhibit 12)
- Ghosh, A. et al. "Generalized Born Model Based on a Surface Integral Formulation" *J. Phys. Chem. B*, (1998) 102, 10983-10990. (Exhibit 13)
- Giver, L. et al., "Directed evolution of a therostable esterase", *Proc. Natl. Acad. Sci. USA* (1998), 95: 12809-12813. (Exhibit 14)

- Gonzales, L. et al., "Crystal structures of a single coiled-coil peptide in two oligomeric states reveal the basis for structural polymorphism", *Nat. Struct. Biol.* (1996), 3: 1002-1100. (Exhibit 15)
- Gough, C. A. et al., "Calculations of the relative free energies of aqueous solvation of several fluorocarbons: A test of the bond potential of mean force correction" *J. Chem. Phys.* (1993), 99: 9103-9110. (Exhibit 16)
- Handel, T. M. et al., "Metal Ion-Dependent Modulation of the Dynamics of a Designed Protein", *Science*, (1993), 261: 879-885. (Exhibit 17)
- Harbury, P. B. et al., "A Switch Between Two-, Three-, and Four- Stretched Coiled Coils in GCN4 Leucine Zipper Mutants", *Science*, (1993), 262, 1401-1407. (Exhibit 18)
- Harpaz, Y. et al., "Volume changes on protein folding", *Structure* (1994), 2: 641-649. (Exhibit 19)
- Hill, C. P. et al., "The structure of granulocyte-colony-stimulating factor and its relationship to other growth factors" *Proc. Natl. Acad. Sci. USA* (1993), 90, 5167-5171. (Exhibit 20)
- Hine, J. et al., "The Intrinsic Hydrophilic Character of Organic Compounds, Correlations in Terms of Structural Contributions", *J. Org. Chem.* (1975), 40: 292-297. (Exhibit 21)
- Hockings, S. C. et al., "Characterization of the ATF/CREB site and its complex with GCN4", *Proc. Natl. Acad. Sci. USA* (1998), 95: 1410-1415. (Exhibit 22)
- Keller, P. et al., "Crystal Structure of a Bzip/dna Complex at 2.2 Å: Determinants of DNA Specific Recognition", *J. Mol. Biol.* (1995), 254: 657-667. (Exhibit 23)
- Kenar, K. T. et al., "A calorimetric characterization of the salt dependence of the stability of the GCN4 leucine zipper", *Protein Sci.* (1995), 4: 1934-1938. (Exhibit 24)
- Konig, P. et al., "The X-ray Structure of the GCN4-Bzip Bound to ATF/CREB Site DNA Shows the Complex Depends on DNA Flexibility", *J. Mol. Biol.* (1993), 233: 139-154. (Exhibit 25)

- Kono, H. et al., "Designing the hydrophobic core of *Thermus flavus* malate dehydrogenase based on side-chain packing", *Protein Eng.* (1998), 11: 47-52. (Exhibit 26)
- Kroll, D. J. et al., "A Multifunctional Prokaryotic Protein Expression System: Overproduction, Affinity Purification, and Selective Detection", *DNA Cell Biol* (1993), 12: 441-53. (Exhibit 27)
- Krylov, D. et al., "A thermodynamic scale for leucine zipper stability and dimerization specificity: e and g interhelical interactions", *EMBO J.* (1994), 13: 2849-2861. (Exhibit 28)
- Lee, B. et al., "Stability of protein structures", *Curr. Opin. Biotech.* (1997), 8: 423-426. (Exhibit 29)
- Lim, K-T. et al., "Molecular Dynamics for Very Large Systems on Massively Parallel Computers: The MPSim program", *J. Comp. Chem.* (1997), 18: 501-521. (Exhibit 30)
- Lubieniski, M. J. et al., "Three-Dimensional Solution Structure and <sup>13</sup>C Assignments of Barstar Using Nuclear Magnetic Resonance Spectroscopy", *Biochemistry* (1994), 33: 8866-8877. (Exhibit 31)
- Lumb, K. J. et al., "Measurement of Interhelical Electrostatic Interactions in the GCN4 Leucine Zipper", *Science* (1995), 268: 436-438. (Exhibit 32)
- Matthews, B. W. "Studies on Protein Stability with T4 Lysozyme", *Adv. Protein Chem.* (1995), 46: 249-295. (Exhibit 33)
- Mendel, D. et al., "Probing Protein Stability with Unnatural Amino Acids", *Science* (1992), 256, 1798-1802. (Exhibit 34)
- Mer, G. et al., "Stabilization of proteins by glycosylation examined by NMR analysis of a fucosylated proteinase inhibitor", *Nat. Struct. Biol.* (1996), 3: 45-53. (Exhibit 35)
- Metallo, S. J. et al., "Distribution of labor among bZIP segments in the control of DNA affinity and specificity", *Chem. Biol.* (1994), 1: 143-151. (Exhibit 36)

- Mohanty, D. et al., "DeNovo Simulations of the Folding Thermodynamics of the GCN4 Leucine Zipper", *Biophys. J.* (1999), 77: 54-69. (Exhibit 37)
- Moitra, J. et al., "Leucine is the Most Stabilizing Aliphatic Amino Acid in the d Position of a Dimeric Leucine Zipper Coiled Coil", *Biochemistry* (1997), 36: 12567-12573. (Exhibit 38)
- Nautiyal, S. et al., "Crystal structure of a designed, thermostable, heterotrimeric coiled coil", *Protein Sci.* (1999), 8: 84-90. (Exhibit 39)
- O'Shea, E. K. et al., "X-ray Structure of the GCN4 Leucine Zipper, a Two-Stranded, Parallel Coiled Coil", *Science* (1989), 254: 538-542. (Exhibit 40)
- O'Shea, E. K. et al., "Evidence that the Leucine Zipper is a Coiled Coil", *Science* (1991), 254: 539-544. (Exhibit 41)
- Paolella, D. N. et al., "DNA Targets for Certain Bzip Proteins Distinguished by an Intrinsic Bend", *Science* (1994), 264: 1130-1133. (Exhibit 42)
- Petka, W. A. et al., "Reversible Hydrogels from Self-Assembling Artificial Proteins", *Science* (1998), 281: 389-392. (Exhibit 43)
- Rennert, O. M. et al., "On the Incorporation of 5', 5', 5'-Trifluoroleucine into Proteins of *E. coli*", *Biochemistry* (1963), 2: 471-476. (Exhibit 44)
- Roux, M. et al., "Fragmentation of Phospholipid Bilayers by Myelin Basic Protein", *Biochemistry* 33: 307 1994. (Exhibit 45)
- Sandberg, W. et al., "Influence of Interior Packing and Hydrophobicity on the Stability of a Protein", *Science* (1989), 245: 54-57. (Exhibit 46)
- Schneider, J. P. et al., "A Designed Buried Salt Bridge in a Heterodimeric Coiled Coil", *J. Am. Chem. Soc.* (1997), 119: 5742-5743. (Exhibit 47)
- Sharma, N. et al., "Efficient introduction of aryl bromide functionality into proteins in vivo", *FEBS Lett.* (2000), 467: 37-40. (Exhibit 48)
- Southern, E. M. "Detection of Specific Sequences among DNA Fragments Separated by Gel Electrophoresis", *J Mol Biol.* (1975), 98:503-517. (Exhibit 49)

- Tannor, D. J. et al., "Accurate First Principles Calculation of Molecular Charge Distributions and Solvation Energies from Ab initio Quantum Mechanics and Continuum Dielectric Theory", *J. Am. Chem. Soc.*, (1994), 116: 11875-11882. (Exhibit 50)
- Thompson, K. S. et al., "Thermodynamic Characterization of the Structural Stability of the Coiled-Coil Region of the Bzip Transcription Factor GCN4", *Biochemistry* (1993), 32: 5491-5496. (Exhibit 51)
- van Hest, J. C. M. et al., "Efficient introduction of alkene functionality into proteins in vivo", *FEBS Lett.*, (1998), 428: 68-70. (Exhibit 52)
- van Hest, J. C. M. et al., "Efficient Incorporation of Unsaturated Methionine Analogs into Proteins in Vivo", *J. Am. Chem. Soc.* (2000), 122: 1282-1288. (Exhibit 53)
- Wendt, H. et al., "Kinetics of Folding of Leucine Zipper Domains", *Biochemistry* (1995), 34: 4091-4107. (Exhibit 54)
- Wigler, M. et al., "DNA-mediated transfer of the adenine phosphoribosyltransferase locus into mammalian cells", *Proc Natl Acad Sci USA* (1979), 76:1373-76. (Exhibit 55)
- Zhang, X-J. et al., "Enhancement of Protein Stability by the combination of point mutations in T4 lysozyme is additive", *Protein Eng.* (1995), 8: 1017-1022. (Exhibit 56)
- Zhang, C. et al., "Asymmetric Synthesis of (S)-5,5,5',5',5'-Hexafluoroleucine", *Helv. Chim. Acta* (1998), 81: 174-181. (Exhibit 57)
- Zhou, Y. et al., "Building a Thermostable Membrane Protein", *J. Biol. Chem.* (2000), 275: 6975-6979. (Exhibit 58)

This statement should be considered because it is submitted before the mailing date of the first Office Action on the merits. In accordance with 37 C.F.R. §1.98(d), copies of each document or other information listed on the enclosed Form 1449 are provided.

No representation is made that a reference is "prior art" within the meaning of 35 U.S.C. §§ 102 and 103 and Applicants reserve the right, pursuant to 37 C.F.R. § 1.131 or otherwise, to establish that the reference(s) are not "prior art." Moreover, Applicants do not represent that the references have been thoroughly reviewed or that any relevance of any portion of a reference is intended.

Consideration of the items listed is respectfully requested. Pursuant to the provisions of M.P.E.P. 609, it is requested that the Examiner return a copy of the attached Form 1449, marked as being considered and initialed by the Examiner, to the undersigned with the next official communication.

No fee is deemed necessary in connection with the filing of this Information Disclosure Statement. However, if any additional fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 50-0306.

Respectfully submitted,



Sarah B. Adriano  
Registration No. 34,470  
Attorney for Applicants  
Mandel & Adriano  
35 No. Arroyo Parkway, Suite 60  
Pasadena, California 91103  
(626)395-7801  
Customer No.: 26941